Stress in Laboratory Juvenile Rabbits: Physiological Indicators

Adina Baias, Alina Bodnariu, Ileana Nichita, Romeo T. Cristina

Banat's University of Agricultural Sciences and Veterinary Medicine from Timişoara, Faculty of Veterinary Medicine Timisoara, 300645- Timisoara, Calea Aradului, 119, Romania

Abstract

The aim of this study was to assess the effect of stress on the main physiologic indicators: body temperature, heart rate, respiratory rate, urinary cortisol and creatinine levels and fecal corticosterone in juvenile rabbits (*Oryctolagus cuniculus*). One seven-week old group-reared rabbits were kept in individual isolation for 24 hours in metabolic cages. This caused the increase at the skin level of the mean body temperature measured, from 31.2°C to 31.5°C, also an increase of the mean heart rate, from 201 beats/min. to 217 beats/min. and an increase of the mean respiratory rate from 47 respirations/min. to 55 respirations/min. At the end of the individual isolation period, the average urinary cortisol/creatinine ratio was of: 9.09 x 10^{-6} and the average value of fecal corticosterone was 557 ng/g. Isolation of group-reared individuals, represents a stress factor that influence the welfare of animals used in scientific experiments, in this respect, fecal corticosterone and urinary cortisol measurements represent an useful non-invasive method for the stress assessment in laboratory animals used for scientific purposes.

Keywords: cortisol, corticosterone, creatinine, *Oryctolagus cuniculus,* stress indicators

1. Introduction

Environmental conditions such as housing and husbandry have a major impact on laboratory animals throughout its life and will therefore influence the outcome of animal experiments. Laboratory rabbits *(Oryctolagus cuniculus)* are conventionally housed individually in cages that are psychologically unstimulating, socially isolating, limiting for exercise and unlikely to be enriching to their quality of life. Group housing has greater potential than single housing for positive enrichment through social interaction, increased exercise, and mental stimulation from the exploration of more complex environments [1]. The young rabbits leave the breeding burrow for the first time at the age of 20 days and start to interact with adults and other juveniles [2]. It is possible that grouping domestic rabbits in a small space could impose stress. Stress in laboratory animals significantly alters normal physiology and metabolism, and therefore increases variation within and between individual animals.

Combined with the between-animal variation in stress perception, this makes stress a major source of experimental error [3, 4]. Persistent stress is accompanied by several adverse effects on most homeostatic mechanisms of the body, including the immune, the endocrine, and the reproductive systems [5]. Stress is therefore generally acknowledged not only as a confounding variable in experimental results, but also as a major cause of suffering in laboratory animals. Refinement of stressful procedures to which laboratory animals are subjected is therefore essential.

In order to achieve this, development of adequate objective methods for the assessment and recognition of stress in laboratory animals is a major challenge in biomedical research [1].

*Corresponding author: Adina Baias,
Email: adina.baias@gmail.com

Stress can be assessed by quantifying different endogenous stress markers, of which the most commonly investigated are corticosteroids.
A stressful stimulus results in an activation of the hypothalamic pituitary adrenal axis, causing a release of corticosteroids from the adrenal cortex [6, 7]. The biologically active corticosteroids are generally cortisol or corticosterone depending on the species. In rats and mice, the predominant corticosteroid is corticosterone.
The concentration of cortisol in blood is widely used as an indicator of stress, although caution is advised, since an increase does not occur with every type of stressor. One has to consider that sample collection, which often involves confinement or handling of animals, may by itself be stressful and may confound the results [7].
Alternatively, several authors have investigated non-invasive sampling procedures such as corticosteroid determinations in the urine and in the feces [6, 8].
In the present study we assess the effect of stress on body temperature, heart rate, respiratory rate, urinary cortisol and creatinine levels and fecal corticosterone in ten juvenile rabbits.

2. Materials and methods

In this study we used 10 rabbits of seven weeks (five males and five females). Animals belonged to a private farm and at the end of the experiment they were sold as pets. Body weight of animals was between 170-410g. In the area where this experiment was conducted, the temperature was between 25-29°C and the relative humidity between 50-55%. Water and food suitable for this species and age category were offered *ad libitum*.
The stress factor was represented by isolation from the group. To achieve this purpose we used metabolic cages, where each rabbit was isolated for 24 hours. Physiological parameters were used for stress assessment. Physiological indicators of stress such as body temperature, heart rate, and respiratory rate, corticosterone in faeces and urinary cortisol/creatinine ratio were determined.
Urinary cortisol and faecal corticosterone reflect average serum cortisol values corresponding to a period of several hours before sampling. Reporting the urinary cortisol/creatinine ratio rather than urinary cortisol alone allows for individual variations in urine concentration.
The body temperature, heart rate and respiratory rate were recorded at the beginning of the study (T_i, $H.r._i$, $F.r._i$), at 12 hours (T_1, $H.r._1$, $R.r._1$) and at 24 hours (T_f, $H.r._f$, $R.r._f$). Urine and faecal samples were collected after 24 hours. Urine samples were acidified with 6M hydrochloric acid solution. All samples were frozen at -20°C. They were processed by enzyme immunoassay, using an Immulite 2000 analyser LabServices, UK.
The eexperiment was conducted in accordance with the requirements of *European Convention for Vertebrate Animal Protection Use in Experiments and Others Experimental Purposes*, adopted in Strasbourg on 18 March 1986 and ratified by the Romanian Law 305/2006.

3. Results and discussion

Minitab 16 for Windows was used for statistical analysis of the data. Non-parametric tests (Mann-Whitney and Pearson correlation tests) were used. Differences were considered statistically significant for *p*-values <0.05.
Values of body temperature, heart rate and respiratory rate are presented in table 1.
During the study the mean values of body temperature, measured at the skin level, increased from 31.2 to 31.5° C.
There was no significant difference between initial values of body temperature and values recorded at 12 and 24 hours following isolation from the group (Mann-Whitney test, all *p*-values >0.08).
Initial heart rate values were significantly lower than values recorded at 24 hours following isolation from the group (Mann-Whitney test, p-value=0.0189). This may indicate that, by the end of the 24 hours, the rabbits anticipated the manipulation stress associated with heart rate measurement. However, values recorded at 12 hours following isolation from the group did not differ significantly from initial values and values recorded at 24 hours (Mann-Whitney test, all *p*-values >0.15).
Initial respiratory rate values were significantly lower than values recorded at 24 hours following isolation from the group (Mann-Whitney test, *p*-value=0.003). Again, this may indicate an anticipation of a stressful situation.

Table 1. Values of body temperature, heart rate and respiratory rate recorded at the beginning of study, at 12 and 24 hours

No.	Body temperature (^{0}C)			Heart rate (no./min)			Respiratory rate (no./min)		
	T_i	T_1	T_f	F.c.$_i$	F.c.$_1$	F.c.$_f$	F.r.$_f$	F.r.$_f$	F.r.$_f$
R_1	30.2	30.5	30.9	210	218	224	44	48	52
R_2	30.3	30.4	30.7	192	194	200	34	38	48
R_3	30.1	30.4	30.6	182	190	198	42	46	52
R_4	30.3	30.5	30.9	202	212	216	54	58	60
R_5	30.0	30.3	30.4	200	208	214	48	50	54
R_6	31.1	31.6	31.9	192	198	208	42	46	52
R_7	33.5	34.1	34.3	204	212	218	56	58	60
R_8	31.5	31.8	32.2	210	218	226	48	56	58
R_9	31.9	32.0	32.3	198	216	228	52	54	60
R_{10}	30.1	30.5	31.4	224	238	242	50	56	62

As respiratory rate was measured by inspection from a standard distance, we may assume that the presence of the assessor represented a stressor for the rabbits. Respiratory rate values recorded at 12 hours following isolation from the group did not differ significantly from initial values and values recorded at 24 hours (Mann-Whitney test, all *p*-values >0.087).

Values of fecal corticosterone and urine cortisol/creatinine ratio are presented in Table 2.

Tabel 2. Values of faecal corticosteron and urine cortisol/creatinine ratio after 24 hours

No.	Faecal corticosteron (ng/g)	Urine cortisol/creatinine *ratio*
R_1	350	3.4×10^{-6}
R_2	320	14.9×10^{-6}
R_3	530	11.8×10^{-6}
R_4	680	11.4×10^{-6}
R_5	340	6.9×10^{-6}
R_6	330	3.2×10^{-6}
R_7	420	8.0×10^{-6}
R_8	510	9.4×10^{-6}
R_9	650	15.4×10^{-6}
R_{10}	440	6.5×10^{-6}

No significant correlations were found between values of urinary cortisol/creatinine *ratio* and values of body temperature, heart rate and respiratory rate (Pearson correlation test, all *p*-values>0.265). There were no significant correlations between values of fecal corticosterone and values of body temperature, heart rate and respiratory rate (Pearson correlation test, all *p*-values>0.057).

The relationship between values of fecal corticosterone and values of respiratory rate recorded at 24 hours following isolation from the group was almost statistically significant, with a positive correlation (Pearson correlation test, *p*-values=0.057, corr=0.618).This may indicate that high circulating levels of cortisol are likely to occur in rabbits which display tachypnoea. However, this assumption needs further testing on larger groups of animals. No significant correlation was identified between values of urinary cortisol/creatinine ratio and values of fecal corticosterone (Pearson correlation test, *p*-values=0.094).

Balcombe and al. [4] suggest that routine handling, venipuncture and orogastric gavage lead to elevations of heart rate, blood pressure and glucocorticoid concentrations. This persists for 30 to 60 minute or more following the event.

Siswanto and al. [10] detected an increase in corticosterone excretion faces eight hours after ACTH injection. However, the increase in fecal corticosterone was only observed after injection of 100 µg/bwt ACTH, compared to the other groups. This indicates that when using quantification of fecal corticosterone for stress assessment, the preceding stress response has to be substantial in order to be detectable in fecal samples. Also, previous observations showed that fecal corticosterone was significantly elevated after surgery but not after single-housing in metabolic cages, which is in accordance with our findings.

4. Conclusions

1. The heart rate and respiratory rate represent accurate, inexpensive and non-invasive indicators of stress in juvenile rabbits.
2. The usefulness of urinary cortisol and fecal corticosterone remains to be demonstrated in this age-group.
3. Measuring these parameters as indicators of adrenocortical activity in animals offers the advantage of simple sampling techniques that do not interfere with the results of the study and enable long term, longitudinal studies.
4. Thus, such methods may represent a valuable tool in a variety of research fields such as animal welfare (handling, housing and transportation of animals) but also in ethological and environmental studies.

Acknowledgements

This work was published during the project "POSTDOCTORAL SCHOOL OF AGRICULTURE AND VETERINARY MEDICINE", POS DRU /89/1.5/S/62371, co-financed by the European Social Fund through the Sectorial Operational Programme for the Human Resources Development 2007-2013.

References

1. Whary, M., Peper, R., Borkowski, G., Wendy, L., Ferguson, F., The effects of group housing on the research use of the laboratory rabbit. Laboratory Animals, 1993 27, p. 330-341.
2. Heiko, Rödel, Raquel, Monclús, Dietrich, Von Holst, Behavioral styles in European rabbits: social interactions and responses to experimental stressors. Physiology and Behavior, 2006, 89, 180-188.
3. Ann, Baldwin, Bekoff, M., Ignoring stress in lab animals could mar research. New Scientist, 2007, 2602, 24-26.
4. Balcombe, J.M, Barnard, N.D, Sandusky, C., Laboratory Routines Cause Animal Stress. Contemporany Topics by the American Association for Laboratory Animal Science, 2004, 43(6), 42-51.
5. Kathleen, Morgan, Tomborg, C., Sources of stress in captivity. Applied Animal Behavior Science, 2007, 102, 262-302.
6. Hau, J.K., Kalliokoski, O., Kristen, Jacobsen, Abelson, K., Interpretation of fecal concentrations of corticosteroids. Laboratory Animals, 2011, 45, 129-130.
7. Möstl, E., Palme, R., Hormones as indicators of stress, Domestic Animal Endocrinology, 2002, 23, 67-74.
8. Laura, Dixon, Jessica, Hardiman, Cooper, J.J., The effects of spatial restriction on the behavior of rabbits (Oryctolagus cuniculus). Journal of Veterinary Behavior, 2010, 5, 302–308.
9. Milot, M.R, James, J.S., Merali, Z., Hélène Plamondon, A refined blood collection method for quantifying corticosterone. Lab Animal, 2012, 41, nr. 3.
10. Siswanto, H., Hau, J., Carlsson, H.E., Goldkuhl, R., Abelson, K.S.P., Corticosterone concentrations in blood and excretion in faeces after ACTH administration in male Sprague-Dawley rats. In vivo, 2008, 22, 435-440